FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 71745/55913

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/856336

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/03830	November 17, 1999	November 17, 1998

TITLE OF INVENTION: **NUCLEIC ACID ISOLATION**

APPLICANT(S) FOR DO/EO/US
Neil James Butt
Christopher Peter Jones

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☒ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☒ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☒ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☒ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Corrected Version of Page 1 of International Publication
PCT/RO/101-Request **PCT/IPEA/401-Demand** **PCT/IPEA/408-Written Opinion** **Letter Response to Written Opinion**
PCT/IPEA/402 **PCT/IB/304** **PCT/IB/308**

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET I
09/856336	PCT/GB99/03830	71745/55913

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO US
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**	
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	40 - 20 =	20	x $18.00	**$360.00**
Independent claims	2 - 3 =	0	x $80.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☒	**$270.00**

TOTAL OF ABOVE CALCULATIONS =	**$1,620.00**
☐ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.	**$0.00**
SUBTOTAL =	**$1,620.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +	**$0.00**
TOTAL NATIONAL FEE =	**$1,620.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐	**$0.00**
TOTAL FEES ENCLOSED =	**$1,620.00**
Amount to be: refunded	$
charged	$

a. ☒ A check in the amount of **$1,620.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **04-1105** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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27026
REGISTRATION NUMBER

May 17, 2001
DATE

NUCLEIC ACID ISOLATION

The present invention relates to a method for isolating nucleic acid, and particularly to a method for isolating plasmid DNA from a plasmid DNA-containing material.

Conventional procedures for the purification of nucleic acid, such as DNA, generally require multiple steps including lysis of source material followed by fractionation steps which may involve column chromatography. Where DNA manipulation is to be carried out, small scale DNA preparations are required routinely, often in large quantities for the purpose of screening DNA from the source cells. These processes are time consuming and labour intensive.

Various methods have been proposed in the purification of such DNA, including a precipitation method in EP-A-0376080, an ultrafiltration method in WO-A-87/07645 and EP-A-0517515 and cationic exchange resins in EP-A-0281390 and EP-A-0366438. A simplified method involving a filter, which is automatable, is disclosed in WO-A-95/02049.

Each of these methods suffers from a disadvantage that a series of steps is required and/or special apparatus is required to achieve sufficient purification of the plasmid DNA. A need therefore arises for a much simpler method involving readily-available apparatus and relatively inexpensive reagents. In a known approach for rapid purification of genomic DNA, RNA or protein, a mixture of phenol, chloroform and guanidine is used (Chomczynski, P. and Sacchi, N., 1987 Anal Biochem. 162: 156; Chomczynski, P., 1993 Biotechniques 15: 532) in which the DNA is extracted into an aqueous phase. This method is unsuitable for isolating plasmid DNA. Moreover, the use of phenol and chloroform is undesirable as these are toxic substances.

The present invention aims to overcome the disadvantages of the prior art and to provide a simplified method for isolating plasmid DNA.

Accordingly, the present invention provides a method for isolating plasmid DNA from DNA containing material which comprises plasmid DNA and genomic DNA, comprising:

(i) extracting the plasmid DNA into a water-immiscible organic solvent capable of supporting plasmid DNA, by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA; optionally separating the organic and aqueous phases of step (i); and

(ii) recovering the plasmid DNA from the organic phase.

Accordingly, the present invention provides a "one step" method which is simple to perform and which requires no specialised laboratory apparatus. It is surprisingly found that this method is capable of extracting plasmid DNA to high purity and with particularly low or zero contamination from genomic DNA which might be present in the plasmid DNA-containing material. In a preferred arrangement, the organic solvent is capable of selectively supporting the plasmid DNA with the exclusion of genomic DNA present in the plasmid DNA-containing material.

The method of the present invention may be performed on a small routine laboratory scale working with solution volumes of microlitres or millilitres. Alternatively, the method may be scaled up even to pilot or industrial scale involving volumes of litres or greater.

In extraction step (*i*), the DNA-containing material is mixed with the reagents under conditions to denature the genomic DNA typically whereby the plasmid DNA is partitioned into an organic

phase and the genomic DNA is partitioned into an aqueous phase. Such conditions include basic conditions or elevated temperature. Suitable elevated temperatures are of at least 65°C and more preferably in the range 70 to 95°C for a time sufficient to denature the plasmid DNA such as from about 30s to about 10mins, preferably around five minutes. Incubation times longer than about 10 minutes at elevated temperature should not adversely affect the plasmid DNA but are undesirable for using the organic solvent. In a preferred arrangement, basic conditions are used in which a base is present. The base is typically a hydroxide such as an alkali metal hydroxide, preferably sodium hydroxide. The base is preferably present at a concentration in the range 100mM to 200mM. Incubation time is usually in the range from about 30s to about 10mins, preferably around five minutes. Excessive incubation under basic conditions can damage the plasmid DNA.

Without wishing to be bound by theory, it is thought that differential solubility between plasmid and genomic DNA under denaturing condition may result in plasmid DNA in an undenatured or reversibly denatured state partitioning into the organic phase. In contrast denatured genomic DNA partitions into the aqueous phase.

The organic solvent must be immiscible with the aqueous phase and preferably comprises an alcohol which may be aliphatic or aromatic and which may be linear or branched chain. The alcohol is preferably a C_3 to C_6 alcohol, more preferably a C_4 to C_6 alcohol and most preferably comprises a butanol such as N-butanol.

The chaotrope may be any normally-recognised chaotrope and is preferably selected from guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate and mixtures thereof. A preferred chaotrope is guanidine hydrochloride. Typically, the

chaotrope is present at a concentration in the range 0.7M to 1.2M, based on the combination of organic solvents, chaotrope water. The concentration of the chaotrope is preferably about 0.9M.

The amount of organic solvent is typically in the range from 20 to 70% based on the volume of the combination of organic solvent, chaotrope or water and is preferably in the range from 35 to 50%, more preferably around 42%.

The exact organic solvent, chaotrope, base and amounts thereof are readily determinable by routine experimentation. Each of these reagents may be mixed with the DNA-containing material in any order or may be premixed prior to addition to the plasmid-containing material. In a convenient arrangement, the organic solvent, chaotrope, base and water are combined to form an extraction mixture. In this arrangement, the extraction step (*i*) comprises mixing the extraction mixture with the DNA-containing material.

At laboratory scale, the step (*ii*) of separating the organic and aqueous phases may be conveniently carried out by allowing the phases to separate or encouraging separation on the basis of density by a short spin in a microcentrifuge. Typically, either the organic or aqueous phase is removed from the other prior to recovery step (*iii*). For example, the organic phase containing the plasmid DNA may be transferred from one container to another by pipette prior to recovery. On a larger scale, removal of one phase from the other could be performed by any conventional method including pumping or running off by gravity one of the two phases.

In one arrangement, recovery step (*iii*) includes precipitation of the plasmid DNA from the organic solvent. For example, the DNA-containing organic phase may be mixed with a precipitating

agent that can precipitate the plasmid DNA from the organic solvent and the precipitated plasmid DNA is separated from the solvent. The precipitated plasmid DNA may also be washed in a washing step. The precipitating agent may comprise an alcohol such as ethanol and may further comprise an acetate salt such as sodium acetate.

The DNA-containing material may comprise any known DNA-containing material such as a bacterial culture which may be lysed or unlysed.

In a further aspect, the present invention provides an extraction mixture for selectively extracting plasmid DNA from a DNA-containing material, which extraction mixture comprises a water-immiscible organic solvent capable of supporting plasmid DNA, a chaotrope and water. The extraction mixture preferably further comprises a base.

The organic solvent, chaotrope, base and amounts thereof are typically those described above.

The present invention will now be described in further detail, by way of example only, with reference to the following Examples.

Example 1

General procedure

Bacterial culture (*E coli* containing pBluescript; 0.5ml) was spun down in an eppendorf tube using a microcentrifuge and the supernatant was discarded. The pellet was resuspended in TE buffer (tris[hydroxymethyl]aminomethane hydrochloride 10mM, EDTA 1mM; pH8.0; 200μl) to form a resuspended pellet containing both genomic and plasmid DNA. An extraction mixture was selected according to the Table below, mixed very well and 0.5ml thereof was added to the resuspended pellet and gently mixed. The eppendorf containing the mixture was spun in a microcentrifuge

for 30 seconds to yield two phases; an upper organic phase and a lower aqueous phase. The organic phase was removed carefully to a fresh eppendorf tube avoiding any contaminating debris. Following measurement of the volume of the removed organic phase, sodium acetate (0.1vols; 3M) and ethanol were added (2vols) to precipitate the plasmid DNA. The eppendorf was spun in a microcentrifuge for 20 minutes and the ethanol supernatant removed. The pellet was rinsed with fresh ethanol (70%; 200μl) and spun for 5 minutes. The ethanol was removed and the pellet dried and resuspended in water (20μl). The resultant plasmid-containing DNA solution could then be assayed by visualisation on an agarose gel and the amount of DNA determined quantitatively by spectrophotometry or by fluorescence.

Table of Extraction Mixtures Tested

CHAOTROPE	NaOH	SOLVENT	PLASMID DNA RECOVERY
GuSCN 0.9M	150mM	N-Butanol 42%	Poor
GuSCN 0.9M	90mM	N-Butanol 42%	Poor
GuSCN 0.9M	200mM	N-Butanol 42%	Poor
GuSCN 0.9M	90mM	N-Butanol 20%	Poor
GuSCN 0.9M	150mM	N-Butanol 20%	Poor
GuSCN 0.9M	200mM	N-Butanol 20%	Good
GuSCN 0.9M	90mM	N-Butanol 70%	No
GuSCN 0.9M	150mM	N-Butanol 70%	No
GuSCN 0.9M	200mM	N-Butanol 70%	No
GuHCl 0.9M	90mM	N-Butanol 42%	Good
GuHCl 0.9M	150mM	N-Butanol 42%	Good
GuHCl 0.9M	200mM	N-Butanol 42%	Good
GuHCl 0.9M	90mM	N-Butanol 20%	OK
GuHCl 0.9M	150mM	N-Butanol 20%	OK
GuHCl 0.9M	200mM	N-Butanol 20%	Poor
GuHCl 0.9M	90mM	N-Butanol 70%	OK
GuHCl 0.9M	150mM	N-Butanol 70%	OK
GuHCl 0.9M	200mM	N-Butanol 70%	Poor
GuHCl 0.9M	90mM	2 methyl propanol 20%	Poor
GuHCl 0.9M	150mM	2 methyl propanol 20%	Poor
GuHCl 0.9M	200mM	2 methyl propanol 20%	Poor
GuHCl 0.9M	90mM	2 methyl propanol 70%	No
GuHCl 0.9M	150mM	2 methyl propanol 70%	No
GuHCl 0.9M	200mM	2 methyl propanol 70%	No
GuHCl 0.9M	90mM	2 methyl propanol 42%	Poor
GuHCl 0.9M	150mM	2 methyl propanol 42%	OK
GuHCl 0.9M	200mM	2 methyl propanol 42%	OK

GuHCl 0.9M	90mM	Butan-2-ol 42%	Poor
GuHCl 0.9M	150mM	Butan-2-ol 42%	OK
GuHCl 0.9M	200mM	Butan-2-ol 42%	Good
GuHCl 0.9M	90mM	Butan-2-ol 20%	Poor
GuHCl 0.9M	150mM	Butan-2-ol 20%	Poor
GuHCl 0.9M	200mM	Butan-2-ol 20%	Poor
Na Perchlorate 0.9M	90mM	N-Butanol 42%	Poor
Na Perchlorate 0.9M	150mM	N-Butanol 42%	Poor
Na Perchlorate 0.9M	200mM	N-Butanol 42%	Poor
Na Perchlorate 0.9M	90mM	N-Butanol 70%	Poor
Na Perchlorate 0.9M	150mM	N-Butanol 70%	Poor
Na Perchlorate 0.9M	200mM	N-Butanol 70%	Poor
Na Perchlorate 0.9M	90mM	N-Butanol 20%	Poor
Na Perchlorate 0.9M	150mM	N-Butanol 20%	Poor
Na Perchlorate 0.9M	200mM	N-Butanol 20%	Poor
Na Perchlorate 0.9M	200mM	2 methyl propanol 20%	OK
Na Perchlorate 0.9M	90mM	2 methyl propanol 70%	OK
Na Perchlorate 0.9M	150mM	2 methyl propanol 70%	Poor
Na Perchlorate 0.9M	200mM	2 methyl propanol 70%	Poor
Na Perchlorate 0.9M	90mM	Butan-2-ol 42%	Poor
Na Perchlorate 0.9M	150mM	Butan-2-ol 42%	Poor
Na Perchlorate 0.9M	200mM	Butan-2-ol 42%	OK
Na Perchlorate 0.9M	90mM	Butan-2-ol 20%	No
Na Perchlorate 0.9M	150mM	Butan-2-ol 20%	No
Na Perchlorate 0.9M	200mM	Butan-2-ol 20%	No

Good	Approximately 1μg DNA recovery
OK	Approximately 200ng DNA recovery
Poor	Just visible on agarose gel electrophoresis

It may be concluded from these results that each recognised chaotrope works and that the guanidine hydrochloride is preferred over the guanidine thiocyanate which is, in turn, preferred over sodium perchlorate in terms of DNA recovery. As to solvents, butanol was found to work best whereas pentanol gave only poor DNA recovery. Ethanol and isopropanol were found not to be water-immiscible. Of the butanols, N-butanol was found to be better than either butan-2-ol or 2 methyl propanol.

Whilst TE was used as the resuspension buffer in the procedure, water could also be used, as well as other resuspension buffers.

Example 2

General procedure for extraction using heat instead of alkaline pH

Bacterial culture (*E coli* containing pBluescript; 0.5ml) was spun down in an eppendorf tube using a microcentrifuge and the supernatant was discarded. The pellet was resuspended in TE buffer (tris[hydroxymethyl]aminomethane hydrochloride 10mM, EDTA 1mM; pH8.0; 200μl) to form a resuspended pellet containing both genomic and plasmid DNA. An extraction mixture was selected according to the Table below, mixed very well and 0.5ml was added to resuspended pellet and gently mixed. The eppendorf tube was then placed in a hot water bath at a temperature in the range 70 to 95°C for five minutes and the contents frequently mixed. Care was taken with the lid of the eppendorf tube because of solvent expansion in the tube. The tube was then rapidly cooled on ice for three minutes, which had the effect of separating the plasmid and genomic DNA. The eppendorf containing the mixture was spun in a microcentrifuge for 30 seconds to yield two phases; an upper organic phase and a lower aqueous phase. The organic phase was removed carefully to a fresh eppendorf tube avoiding any contaminating debris. Following measurement of the volume of the removed organic phase, sodium acetate (0.1vols; 3M) and ethanol were added (2vols) to precipitate the plasmid DNA. The eppendorf was spun in a microcentrifuge for 20 minutes and the ethanol supernatant removed. The pellet was rinsed with fresh ethanol (70%; 200μl) and spun for 5 minutes. The ethanol was removed and the pellet dried and resuspended in water (20μl). The resultant plasmid-containing DNA solution could then be assayed by visualisation on an agarose gel and the amount of DNA determined quantitatively by spectrophotometry or fluorescence.

Results comparable to those of Example 1 were obtained although yields were slightly lower and minor contamination with genomic DNA was observed.

AMENDED CLAIMS (Article 34 PCT)

1. A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising the steps of:

(i) extracting the plasmid DNA into butanol by mixing the material with butanol, a chaotrope, and water under conditions to denature the genomic DNA; and

(ii) recovering the plasmid DNA from the butanol.

2. The method of claim 1, wherein the conditions to denature the DNA comprise basic conditions or a temperature of at least 65°C.

3. The method of claim 2, wherein the conditions to denature the DNA comprise basic conditions in which a base is present.

4. The method of any preceding claim, wherein the butanol is n-butanol, 2-methylpropanol, or butan-2-ol.

5. The method of any preceding claim, wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate, and mixtures thereof.

6. The method of claim 6, wherein the chaotrope comprises guanidine hydrochloride.

7. The method of any one of claims 3 to 6, wherein the base comprises a hydroxide.

8. The method of claim 7, wherein the hydroxide comprises sodium hydroxide.

9. The method of any one of claims 3 to 8, wherein the butanol, the chaotrope, the base and the water are combined to form an extraction mixture, and extraction step (i) comprises mixing the extraction mixture with the plasmid DNA-containing material.

10 The method of any preceding claim, wherein the amount of butanol is in the range from 20 to 70% based on the volume of the combination of butanol, chaotrope and water.

11. The method of claim 10, wherein the amount of the butanol is in the range from 35 to 50%.

12. The method of claim 11, wherein the amount of the butanol is about 42%.

13. The method of any preceding claim, wherein the chaotrope is present at a concentration of from 0.7M to 1.2M based on the combination of butanol, chaotrope and water.

14. The method of claim 13, wherein the concentration of the chaotrope is about 0.9M.

15. The method of any preceding claim, wherein the recovery step (ii) comprises mixing the DNA-containing butanol phase with a precipitating agent that can precipitate the plasmid DNA from the butanol, and separating the precipitated plasmid DNA from the butanol.

16. The method of claim 15, wherein the recovery step (ii) further comprises a washing step in which the precipitated plasmid DNA is washed.

17. The method of claim 15 or claim 16, wherein the precipitating agent comprises an alcohol.

18. The method of claim 17, wherein the alcohol is ethanol.

19. The method of any one of claims 15 to 18, wherein the precipitating agent further comprises an acetate salt.

20. The method of claim 19, wherein the acetate salt comprises sodium acetate.

21. The method of any preceding claim, which further comprises a step of separating the organic and aqueous phases of step (i) prior to recovering the plasmid DNA.

22. The method of claim 21, wherein the step of separating the organic and aqueous phases further comprises centrifugation of the mixture formed in step (i) to facilitate separation of the mixture into the organic and aqueous phases.

23. The method of any preceding claim, wherein the DNA-containing material comprises a lysed or unlysed bacterial culture.

24. An extraction mixture for selectively extracting plasmid DNA from a DNA-containing material, which extraction mixture comprises butanol, a chaotrope, and water.

25. The extraction mixture of claim 24, which further comprises a base.

26. The extraction mixture of claim 25, wherein the base comprises a hydroxide.

27. The extraction mixture of claim 26, wherein the hydroxide comprises sodium hydroxide.

28. The extraction mixture of any one of claims 24 to 27, wherein the butanol is n-butanol, 2-methylpropanol, or butan-2-ol.

29. The extraction mixture of any one of claims 24 to 28, wherein the butanol constitutes from 20 to 70% based on the volume of the extraction mixture.

30. The extraction mixture of claim 29, wherein the butanol constitutes from 35 to 50 % of the extraction mixture.

31. The extraction mixture of claim 30, wherein the butanol constitutes about 42% of the extraction mixture.

32. The extraction mixture of any one of claims 24 to 31, wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate, and mixtures thereof.

33. The extraction mixture of claim 32, wherein the chaotrope comprises guanidine hydrochloride.

34. The extraction mixture of any one of claims 24 to 33, wherein the concentration of chaotrope in the extraction mixture is from 0.7M to 1.2M.

35. The extraction mixture of claim 34, wherein the concentration of the chaotrope in the extraction mixture is about 0.9M.

DIKE, BRONSTEIN, ROBERTS & CUSHMAN
INTELLECTUAL PROPERTY GROUP OF
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Docket No. 71745-55913

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed at 201) below or an original, first and joint inventor (if plural names are listed at 201-206 below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: NUCLEIC ACID ISOLATION

which is described and claimed in:

☐ the specification attached hereto.

☐ the specification in U.S. Application Serial Number _____, filed on May 17, 2001.

☐ the specification in PCT international application Number, filed on _____; and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign/PCT Applications and Any Priority Claims Under 35 U.S.C. §119:			
Application No.	**Filing Date**	**Country**	**Priority Claimed Under 35 U.S.C. §119?**
			☐YES ☐NO
			☐YES ☐NO
			☐YES ☐NO

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below, and, insofar as the subject matter of each of the claims of this application is

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature of Inventor **201** Neal Bartl	Signature of Inventor **202** C Peter Jones
Date: 15 July 2001	Date: 5/8/01.
Signature of Inventor **203**	Signature of Inventor **204**
Date:	Date:
Signature of Inventor **205**	Signature of Inventor **206**
Date:	Date:

not disclosed in that/those prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 CFR §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Applications or PCT International Applications Designating the U.S- Benefit Under 35 U.S.C. §120				
U.S. Applications		**Status (Check One)**		
Application Serial No.	**U.S. Filing Date**	**atented**	**Pending**	**Abandoned**
PCT Applications Designating the U.S.				
Application No.	**Filing Date**	**U.S. Serial No. Assigned**		
PCT/GB99/03830	11/17/99			

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S) (35 U.S.C. §119(e))

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Applicant	**Provisional Application Number**	**Filing Date**

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(14)

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